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High ceftazidime hydrolysis activity and porin OmpK35 deficiency contribute to the decreased susceptibility to ceftazidime/avibactam in KPC-producing *Klebsiella pneumoniae*

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Objectives: To investigate mechanisms for the decreased susceptibility to ceftazidime/avibactam in KPC-producing *Klebsiella pneumoniae* (KPC-KP).

Methods: A total of 24 isolates, 8 each with ceftazidime/avibactam MICs of 4–8, 1–2 and ≤0.5 mg/L, were randomly selected from 214 clinical isolates of KPC-KP, and the β-lactamase hydrolysis activity and porin expression profiles were determined. Plasmid profile and relative expression and copy number of the bla_{KPC} gene were also analysed.

Results: Ceftazidime/avibactam MIC_{50} and MIC_{90} were 2 and 4 mg/L, respectively, for the 214 KPC-KP isolates. The hydrolysis activities of nitrocefin and ceftazidime in both of the ceftazidime/avibactam MIC 4–8 and 1–2 mg/L groups were significantly higher than those of the MIC ≤0.5 mg/L group, while the hydrolysis activities were 4–4.6-fold higher in the MIC 4–8 mg/L group than in the other two groups when 4 mg/L avibactam was added. The relative expression and copy number of the bla_{KPC} gene in the MIC 4–8 mg/L group were 4.2–4.8-fold higher than in the other two groups. Meanwhile, SDS-PAGE showed that all isolates in the two groups with MIC ≥1 mg/L lacked OmpK35, which had either an early frameshift with a premature stop codon ($n = 15$, ST11) or overexpression of the negative regulation genes, *micF* and *ompR* ($n = 1$, ST15), whereas OmpK35 and OmpK36 could both be observed in all isolates with MIC ≤0.5 mg/L.

Conclusions: Decreased ceftazidime/avibactam susceptibility in KPC-KP clinical isolates is caused by high ceftazidime hydrolysis activity and OmpK35 porin deficiency and the majority of isolates belong to ST11.

Introduction

The emergence and global dissemination of carbapenem-resistant *Klebsiella pneumoniae* pose a significant therapeutic challenge to public health. The production of carbapenemases is a major contributor to carbapenem resistance in *K. pneumoniae*, with *K. pneumoniae* carbapenemases (KPCs) being the most prevalent cause in China as well as other regions of the world.[1] Epidemiological studies show that both in China and other countries, ∼70.6%–92.0% of carbapenem-resistant *K. pneumoniae* produce KPC.[2–4] KPC-producing *K. pneumoniae* (KPC-KP) may be resistant to all classes of clinically available antimicrobial agents, except colistin and tigecycline.[5] Owing to how widespread KPC-KP is, there is an urgent need for new antibiotics to treat infections caused by these XDR clinical isolates.

Avibactam is a new non-β-lactam, β-lactamase inhibitor, with potent inhibitory activity for KPC, distinguishing it from other traditional β-lactamase inhibitors.[6] The addition of avibactam to ceftazidime considerably decreases the MICs of ceftazidime by 128-fold for KPC-KP.[7,8] In February 2015, the FDA formally approved the ceftazidime/avibactam combination for the treatment of complicated urinary tract infections and adult abdominal infections. As a result, ceftazidime/avibactam may prove to be a valuable addition to the limited antibiotic option against infections caused by KPC-KP.

Carbapenem resistance in *K. pneumoniae*, however, is mediated through multiple mechanisms, in addition to the production of carbapenemases, such as KPCs, NDM-1, VIM, IMP or OXA-48, including decreased outer membrane permeability and expression of ESBLs and plasmid-encoded AmpC enzymes.[1] Owing to deletions interrupting the coding sequence or possibly negative regulation by OmpR and the small non-translated antisense RNA micF,[9,10] OmpK35 porin deficiency has become common in ESBLs and KPC-KP and is associated with increased resistance to hydrophilic antimicrobial agents, such as ceftazidime and other β-lactams.[11] Therefore, inhibition of β-lactamases may not provide a 'one-size-fits-all' therapeutic option against KPC-KP.

Recently, small portions of KPC-KP isolates with significantly decreased susceptibility (up to an MIC 8/4 mg/L) to ceftazidime/avibactam (FDA resistance breakpoints, ≥16/4 mg/L) have been detected across the world.[8] Additionally, a KPC-3-producing *K. pneumoniae* isolate has been reported to be resistant to ceftazidime/avibactam with an MIC 32/4 mg/L and an underlying resistance mechanism still under investigation.[12] This study aims to investigate the mechanisms for decreased susceptibility to ceftazidime/avibactam in a set of KPC-KP clinical isolates from Shanghai.

Materials and methods

Selection of clinical isolates

Non-duplicate clinical isolates of *K. pneumoniae* were routinely recovered from inpatients admitted to Huashan Hospital, Fudan University in Shanghai, China. MICs were determined for ceftazidime and ceftazidime/avibactam using the CLSI reference broth microdilution method for *K. pneumoniae* clinical strains, which had been screened for carbapenem resistance with the CLSI reference disc diffusion method.[13] Avibactam was purchased from MedChem Express (NJ, USA) and was tested at a fixed concentration of 4 mg/L.[13] Comparator agents included cefotaxime, aztreonam, cefepime, piperacillin/tazobactam, meropenem, imipenem, levofloxacin, amikacin, tigecycline and colistin. Quality control strains tested concurrently included *Escherichia coli* ATCC 25922, ATCC 35218 and *K. pneumoniae* ATCC 700603. MICs were interpreted according to CLSI breakpoints,[13] with the exception of ceftazidime/avibactam where MICs were interpreted using the interpretative criteria according to the FDA[14] and tigecycline and colistin where EUCAST breakpoints were applied.[15]

Isolates with non-susceptibility to either of the two carbapenems (imipenem and meropenem) were collected and analysed by PCR for bla_{KPC}, as well as bla_{IMP}, bla_{NDM}, bla_{OXA-48} and bla_{VIM}.[16] A total of 1058 clinical isolates of *K. pneumoniae* were collected during 2014, of which 246 isolates (23.3%) displayed non-susceptibility to carbapenems. A total of 214 clinical isolates carrying bla_{KPC} alone were included. Twenty-four KPC-KP isolates were selected for further investigation of decreasing susceptibility mechanisms according to the range of ceftazidime/avibactam MICs. Of these, eight with ceftazidime/avibactam MICs 4–8 mg/L, eight with MICs 1–2 mg/L and eight with MICs ≤0.5 mg/L were randomly chosen.

Homology analysis of selected isolates

MLST with seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*) was performed for the selected 24 isolates.[17] PFGE analysis was also performed with XbaI-digested DNA (http://www.cdc.gov/pulsenet/pathogens/pfge.html).

Screening for β-lactamases

PCR detection of β-lactamase genes encoding ESBLs (bla_{CTX-M}, bla_{SHV} and bla_{TEM}) and plasmid-borne AmpC β-lactamases (bla_{ACC}, bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{FOX} and bla_{MOX}) was performed for the selected isolates as previously described.[16] PCR amplicons were sequenced and compared with sequences available in the GenBank database using BLAST searches.

Plasmid analysis

Plasmid DNA was extracted from the selected 24 isolates and was introduced by electroporation into *E. coli* DH10B.[18] Putative transformants were selected on LB agar containing 50 mg/L ampicillin. PCR screening for bla_{KPC} was employed for selection of transformants. The plasmid sizes of the isolates and transformants were confirmed by S1-PFGE and the particular plasmid harbouring bla_{KPC} was characterized by PCR-based replicon typing.[19] The genetic environment around bla_{KPC} was determined through crossing PCR as previously described.[20]

Plasmids from transformants were extracted using the QIAGEN Plasmid Midi Kit (Qiagen, Hilden, Germany). For RFLP analysis, plasmids were digested with EcoRI endonuclease and restriction fragments were separated by electrophoresis in 0.8% agarose gel. The genetic relatedness among plasmids was determined by the number of band differences observed in the RFLP profiles. RFLP profiles that differed by ≤3 bands were considered to be within the same restriction pattern type.

The representative bla_{KPC}-carrying plasmids were selected for next-generation sequencing. Ten nanograms of plasmid DNA were employed to prepare barcoded libraries with the Ion Plus Fragment Library kit (Life Technologies, Carlsbad, CA, USA). Sequencing was carried out using Ion318 TM chips on the Ion PGM System (PGM^{TM}, Life Technologies) and with the Ion PGM^{TM} Sequencing 400 kit. The Lasergene sequence analysis software system was used to align the sequence.

Efflux pump inhibitor tests

For these 24 isolates, MICs of ceftazidime/avibactam in combination with PABN (25 mg/L),[21] an inhibitor of resistance–nodulation–division (RND) pumps of Enterobacteriaceae, were determined. A 4-fold decrease in MIC after addition of PABN was considered significant.[21]

β-Lactamase hydrolysis activity

The hydrolysis activity of β-lactamase was monitored at 482 or 260 nm, respectively, using crude supernatants of sonicated bacterial cells at 37°C, with 100 μM nitrocefin or ceftazidime as the substrate in the absence or presence of avibactam (4 mg/L).[22] One unit of enzyme activity was defined as the amount of enzyme that hydrolysed 1 nmol of substrate per min.

Determination of gene expression

Quantitative real-time PCR was employed to assess the transcriptional expression level of bla_{KPC}, *acrB*, *ramA*, *ompK35*, *ompK36*, *ompR* and *micF* in all 24 isolates, as previously described.[9,23] All amplifications were carried out in triplicate from three different RNA preparations. The copy number of bla_{KPC} was measured relative to an internal *K. pneumoniae* housekeeping gene, *rpoB*, as previously described.[24] Standard curves were generated for both the target (bla_{KPC}) and the endogenous control (*rpoB*) using 10-fold dilutions of template DNA at known concentrations.

Analysis of outer membrane proteins

The coding sequences of *ompK35* and *ompK36*, with their respective promoter regions were amplified as previously described.[9] Outer membrane proteins were isolated according to the rapid procedure of Carlone *et al.*[25] and separated by SDS-PAGE.

OmpK35 complementation assays

The *ompK35* gene was amplified from a clinical isolate of SH1496 with ceftazidime/avibactam MIC 0.5 mg/L and cloned into pBad33, an arabinose induction expression vector with apramycin resistance, yielding the recombinant pBad33-ompK35. For complementation, competent cells of clinical isolates were prepared according to standard methods,[26] and recombinant plasmids were introduced by electroporation. Transformants were selected on LB agar containing 50 mg/L of apramycin. The expression of *ompK35* in transformants was induced by 0.2% arabinose (weight/volume) confirmed by real-time PCR and SDS-PAGE analysis.

Results

Antibiotic susceptibility profiles of KPC-KP isolates

KPC-KP was highly resistant to all β-lactams tested, with the exception of ceftazidime/avibactam ($MIC_{50/90}$, 2/4 mg/L) (Table S1, available as Supplementary data at *JAC* Online). Of 214 clinical isolates, 8 (3.7%) and 55 (25.7%) isolates displayed MICs of 8 and 4 mg/L, respectively (Figure S1). Among other antimicrobial agents, only tigecycline ($MIC_{50/90}$, 1/4 mg/L) and colistin ($MIC_{50/90}$, 1/1 mg/L) exhibited excellent activity against these isolates.

Characterization of selected isolates

All 24 isolates produced KPC-2 and various ESBLs genes were also detected (Table 1). PFGE patterns of clinical isolates with ceftazidime/avibactam MIC ≥1 mg/L differed significantly from those with MIC ≤0.5 mg/L. Isolates with MIC ≥1 mg/L were divided into four major phylogenetic groups, sharing ∼70% PFGE pattern similarity (Figure S2). Except for one ST15 isolate, the other 15 isolates with ceftazidime/avibactam MIC ≥1 mg/L were ST11, whereas the remaining isolates with MIC ≤0.5 mg/L proved to be non-ST11 with various STs (Table 1 and Figure S2).

β-Lactamases hydrolysis activity

The hydrolysis activity of nitrocefin and ceftazidime in both of the ceftazidime/avibactam MIC 4–8 and 1–2 mg/L groups was significantly higher than that of the MIC ≤0.5 mg/L group ($P < 0.05$) (Figure 1). Though the addition of avibactam (4 mg/L) decreased the hydrolysis activity of nitrocefin and ceftazidime in all isolates, the hydrolysis activity in the MIC 4–8 mg/L group was 4–4.6-fold higher than that in the other two groups ($P < 0.05$) (Figure 1). No statistically significant difference in hydrolysis activity was observed between the MIC 1–2 and ≤0.5 mg/L groups with the addition of avibactam.

The relative expression and copy number of the bla_{KPC} gene in the MIC 4–8 mg/L group were 4.2–4.8-fold higher than in the other two groups (Figure 1). The selected 24 isolates possessed one to three plasmids. The majority of clinical isolates ($n = 7$) with MIC 4–8 mg/L harboured a 170 kb bla_{KPC}-carrying plasmid, while smaller bla_{KPC}-carrying plasmids of ∼80–130 kb were detected in 14 isolates of the other two groups (Table S2). Though two isolates in the MIC 1–2 mg/L group harboured a 170 kb bla_{KPC}-carrying plasmid, the relative expression and copy number of the bla_{KPC} gene were still ∼4-fold lower than those in the MIC 4–8 mg/L group. RFLP analysis revealed that larger bla_{KPC}-carrying plasmids of 160–170 kb in size all shared a type A restriction pattern, irrespective of ceftazidime/avibactam MIC levels (Table S2). Besides, a total of six bla_{KPC}-carrying plasmids with different EcoRI-digestion patterns, two from each group, were selected for next-generation sequencing and only one copy of the bla_{KPC} gene was spotted on all of the plasmids. Though two isolates of the ceftazidime/avibactam MIC ≤0.5 mg/L group had a deletion in the region upstream of the bla_{KPC} gene, no significant changes on the expression of bla_{KPC} was observed when compared with other isolates in this group.

Role of the AcrAB efflux pump in ceftazidime/avibactam susceptibility

The addition of PABN did not decrease ceftazidime/avibactam MIC by more than 2-fold, indicating a lack of PABN efflux inhibition on ceftazidime/avibactam combination activity in all isolates (Table 1). No statistically significant difference in the level of *acrB* and *ramA* expression was observed among these three groups (Figure S3).

Porin gene expression and sequence analysis

The expression of *ompK35* and *ompK36* showed a 28.5-fold decreased level of *ompK35* mRNA in the two groups with MIC ≥1 mg/L when compared with the MIC ≤0.5 mg/L group, whereas *ompK36* transcription was similar in each group (Figure S3). For the ST15 isolate with ceftazidime/avibactam MIC 1 mg/L, the relative gene expression of *micF* and *ompR* was also 7.2–8-fold higher than the average of the MIC ≤0.5 mg/L group.

The *ompK35* sequence of the 15 ST11 isolates had an A deletion after 85 bp when compared with the reference sequence of GenBank accession number JX310555, resulting in an early frameshift and a premature stop codon following the amino acid 62 codon, producing a truncated protein that was non-functional (Table 1), while the other nine non-ST11 isolates shared an identical *ompK35* sequence with the reference strain. Various amino acid mutations were observed in OmpK36 along with different STs (Table 1). No significant change in promoter region or ribosome binding site of *ompK35* and *ompK36* was observed.

SDS-PAGE analysis showed that all 16 *K. pneumoniae* isolates with MIC ≥1 mg/L lacked OmpK35 porin, but retained normal levels of OmpK36 and OmpA, whereas all three outer membrane protein bands, OmpK36, OmpK35 and OmpA could be observed in the remaining MIC ≤0.5 mg/L isolates (Figure 2).

Functional restoration of OmpK35

The restoration of functional OmpK35 resulted in 2–4-fold decreases in the MICs of ceftazidime alone and the ceftazidime/avibactam combination for the selected isolates, while no significant reduction in avibactam MICs was observed (Table S3). SDS-PAGE analysis confirmed that the missing OmpK35 was restored in all transformants (Figure 2).

Discussion

The present data showed that ceftazidime/avibactam displayed potent activity against KPC-KP clinical isolates, with ceftazidime/avibactam MIC_{50} and MIC_{90} of 2 and 4 mg/L, respectively. However, eight (3.7%) isolates displaying MICs of 8 mg/L were detected, which approached the FDA ≥16/4 mg/L ceftazidime/avibactam resistance breakpoint for Enterobacteriaceae. A recent

Table 1. Characteristics of 24 KPC-KP isolates with various levels of ceftazidime/avibactam susceptibility

		MIC (mg/L)							Porin sequence modifications		
										OmpK36[c]	
Strain	β-Lactamase[a]	AVI	CAZ	CAZ/AVI	CAZ/AVI+25 mg/L PABN	MEM	IPM	ST	OmpK35[b]	reference strain	amino acid variations
MIC 4–8 mg/L											
HS1338	KPC-2, CTX-M-65	>1024	512	8	8	>128	128	11	Ala62fsX	JX291114	–
HS1397	KPC-2, CTX-M-65	256	256	8	4	>128	128	11	Ala62fsX	JX291114	–
HS1617	KPC-2, CTX-M-65, SHV-12	1024	128	8	8	>128	128	11	Ala62fsX	JX291114	–
HS1851	KPC-2, CTX-M-14	256	256	8	4	>128	64	11	Ala62fsX	JX291114	–
HS1343	KPC-2, CTX-M-65, SHV-12	256	256	4	4	>128	64	11	Ala62fsX	JX291114	–
HS1621	KPC-2, CTX-M-65	1024	512	4	4	>128	128	11	Ala62fsX	JX291114	Leu167Val
HS1876	KPC-2, CTX-M-14	512	256	4	4	>128	32	11	Ala62fsX	JX291114	–
HS2281	KPC-2, CTX-M-65	>1024	128	4	2	>128	64	11	Ala62fsX	JX291114	Leu167Val
MIC 1–2 mg/L											
HS1257	KPC-2, CTX-M-65	512	128	2	1	32	32	11	Ala62fsX	JX291114	Gly136 and Asp137 del
HS1386	KPC-2, CTX-M-65	512	64	2	1	128	128	11	Ala62fsX	JX291114	–
HS1507	KPC-2, CTX-M-65, DHA-1	1024	128	2	2	128	64	11	Ala62fsX	JX291114	–
HS1583	KPC-2	256	64	2	1	32	64	11	Ala62fsX	JX291114	Gly136 and Asp137 del
HS923	KPC-2, CTX-M-65	512	16	1	1	64	32	11	Ala62fsX	JX291114	–
HS945	KPC-2, CTX-M-65	1024	64	1	1	128	64	11	Ala62fsX	JX291114	–
HS971	KPC-2, CTX-M-65	512	32	1	1	128	128	11	Ala62fsX	JX291114	–
HS1368	KPC-2	1024	16	1	0.5	64	32	15	–	KT276272	–
MIC ≤0.5 mg/L											
HS1299	KPC-2	512	8	0.25	0.25	2	4	1764	–	KT276272	184–185 del and 307–314 ins
HS1390	KPC-2	512	32	0.5	0.5	2	8	43	–	KT276272	[d]
HS1496	KPC-2	256	16	0.5	0.25	1	2	15	–	KT276272	–
HS1542	KPC-2, CTX-M-14	512	8	0.5	0.25	2	8	15	–	KT276272	–
HS1699	KPC-2	256	8	0.25	0.25	2	4	1764	–	KT276272	184–185 del and 307–314 ins
HS1711	KPC-2	512	16	0.5	0.5	1	8	290	–	KT276272	Tyr137Thr
HS1713	KPC-2	512	16	0.5	0.5	2	8	290	–	KT276272	Tyr137Thr and Leu165Val
HS1754	KPC-2, CTX-M-14	512	16	0.125	0.25	2	8	15	–	KT276272	Tyr137Thr

AVI, avibactam; CAZ, ceftazidime; MEM, meropenem; IPM, imipenem; fsX, frameshift resulting in a premature stop codon; –, no modification; del, deletion; ins, insertion.

[a]Only KPC and ESBL enzymes are listed.

[b]Predicted translational modifications of OmpK35 were based on the reference sequence (GenBank accession number JX310555) from an ST11 strain isolated in our hospital. Sequences of OmpK35 in the present study have been submitted to GenBank under accession numbers KX528024–KX528047.

[c]Predicted translational modifications of OmpK36 were based on two reference sequences (GenBank accession numbers JX291114 and KT276272). The former was from an ST11 strain isolated in our hospital, whereas another was an ST15 strain from Taiwan. Sequences of OmpK36 in the present study have been submitted to GenBank under accession numbers KX528048–KX528070.

[d]Unable to generate a quality PCR product for sequencing despite multiple attempts.

global surveillance programme, which included 476 clinical isolates of KPC-producing Enterobacteriaceae (with 90% being *K. pneumoniae*), also spotted 10 (2.1%) isolates with ceftazidime/avibactam MIC 8 mg/L.[8]

This study showed that higher ceftazidime/avibactam MICs were partially due to higher ceftazidime hydrolysis activity, as ceftazidime hydrolysis activity in the MIC 4–8 mg/L group was 4-fold higher than that in the other two groups ($P < 0.05$), as was the relative gene expression and copy number levels of bla_{KPC} in the MIC 4–8 mg/L group. As no significant changes in the surrounding upstream genetic elements of bla_{KPC} were observed, the high copy number of bla_{KPC} might be attributed to an increased copy number of bla_{KPC}-carrying plasmids.[24,27] As bla_{KPC} and ESBLs genes were located on the same plasmid, the higher hydrolytic activity in the MIC 4–8 mg/L group might also be attributed to the higher expression of bla_{KPC} as well as other ESBLs genes. A recent study has also identified three isolates of KPC-producing *Enterobacter* spp. with ceftazidime/avibactam MICs of 8–32 mg/L.[28] These isolates produced copious amounts of AmpC β-lactamase, as well as KPC enzymes, which simply overwhelmed the inhibitor. As observed in

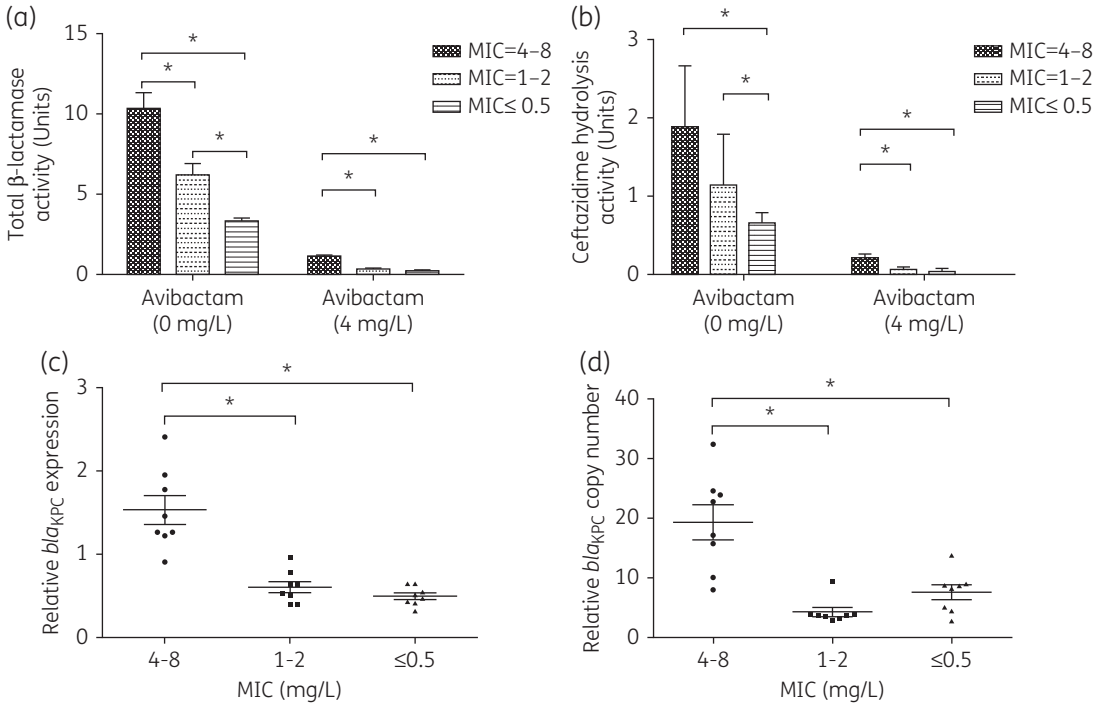

Figure 1. Enzymatic assays, relative bla_{KPC} expression level and gene copy number in selected isolates. (a) Total β-lactamase activity for bacterial lysates was measured using the chromogenic substrate nitrocefin, with or without avibactam at 4 mg/L. (b) Ceftazidime hydrolysis activity of different bacterial lysates was determined with or without avibactam at 4 mg/L. One unit of enzyme activity was defined as the amount of enzyme that hydrolysed 1 nmol of substrate per min. Data are the average of results obtained from three independent experiments and are presented as mean ± SD. (c) Relative bla_{KPC} expression level. (d) Relative bla_{KPC} gene copy number in selected isolates. *$P < 0.05$, Student's *t*-test with Bonferroni correction.

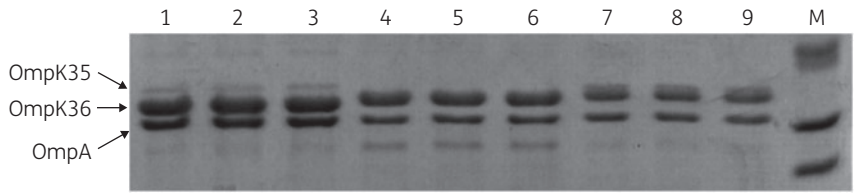

Figure 2. Outer membrane protein profiles of representative clinical isolates of KPC-KP. Lanes 1–3: HS1496, HS1699 and HS1713, with ceftazidime/avibactam MIC ≤0.5 mg/L. Lanes 4–6: HS1617, HS2281 and HS1257, with ceftazidime/avibactam MIC 8, 4 and 2 mg/L, respectively. Lanes 7–9: HS1617, HS2281 and HS1257, with functional restoration of OmpK35. Lane M: protein markers of 45, 33 and 26 kDa.

the hydrolysis activity tests, high amounts of KPC β-lactamase because of bla_{KPC} high-level expression may overwhelm the inhibition of the addition of avibactam (4 mg/L) and produce high ceftazidime/avibactam MICs in KPC-KP clinical isolates.

This study indicated that OmpK35 porin deficiency also contributed to the decreased susceptibility to ceftazidime/avibactam in KPC-KP. It has been reported that OmpK35 played a significant role in ceftazidime susceptibility in *K. pneumoniae* and that MIC reductions by OmpK35 restoration were much higher than that caused by OmpK36.[29] However, no significant reduction in the MICs of avibactam was observed after the restoration. Previous studies have demonstrated that pores formed by OmpK35 were not the major channel through which avibactam penetrates to the periplasm of *K. pneumoniae*.[30] The increase of ceftazidime/avibactam MICs in KPC-KP would best be attributed to the permeability barrier to ceftazidime from the lack of OmpK35.

OmpK35 deficiency in ST11 isolates was attributed to an early frameshift and a premature stop codon in coding sequence, while the decreased expression of *ompK35* in the ST15 isolate with MIC 1 mg/L seemed due to the negative regulation of micF and OmpR. The antisense RNA micF plays a central role in the overall transcriptional regulation of *ompF* (*ompK35*) expression and OmpR negatively regulates the expression level of *ompK35* directly or via micF.[31] In addition, a wide variety of OmpK36 porin profiles were observed among the selected KPC-KP isolates. Except for the substitution of Leu167Val, other amino acid changes have already been reported.[32–34] As previously described in Greece,[33] there was also a strong correlation between OmpK36 alleles and profiles with specific lineages (STs) of *K. pneumoniae* in the current study.

ST11 is the dominant clone of KPC-KP in China,[35] and 15 of 16 isolates with ceftazidime/avibactam MIC ≥1 mg/L were confirmed to be ST11 in the current study. ST11 isolates, with the

increased gene expression of bla_{KPC} and probably the co-production of ESBLs, possessed a higher level of β-lactamase hydrolysis activity when compared with non-ST11 isolates. Higher β-lactamase hydrolysis activity and loss of OmpK35 may contribute to the success of ST11 *K. pneumoniae*. This may help to explain why the ST11 clone is the most prevalent cause of MDR *K. pneumoniae* infections in China.

This study indicated that hydrolysis activity and loss of OmpK35 are significant factors for differentiating the susceptibility of ceftazidime/avibactam in KPC-KP. Our data also provide important cautionary notes as clinicians begin to integrate the use of ceftazidime/avibactam into clinical practice. As β-lactamases overexpression may overwhelm the inhibitor component, ceftazidime/avibactam should be dosed to maximize pharmacokinetic and pharmacodynamics parameters when the MIC is at the higher end of the susceptible range. Further clinical evidence is still needed to recommend confidently such a cephalosporin/β-lactamase inhibitor combination approach in a broad range of clinical situations.

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Transparency declarations

None to declare.

Supplementary data

Tables S1–S3 and Figures S1–S3 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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